Study on the mass spectrometric identification of peptide metabolites from dietary proteins in rat bloodstream

盛, 暁静

出版情報：Kyushu University, 2021, 博士（農学）, 課程博士
バージョン：
権利関係：

Name : Sheng Xiaojing

Title : Study on the mass spectrometric identification of peptide metabolites from dietary proteins in rat bloodstream

(ラットにおけるタンパク質由来ペプチドの血中産生挙動に関する研究)

Category：Kou

Thesis Summary

Dietary protein as an essential macronutrient in our daily life provide high impact on nutritional value and health promoting effects against lifestyle-related diseases, such as hyperlipidemia, diabetes, and obesity. Moreover, protein-derived fragments, i.e., peptides, are also demonstrated to elicit *in vivo* hypolipidemic, anti-hypertensive and anti-diabetic effects. Meanwhile, in the gastrointestinal tract, proteins are degraded by digestive proteases and peptidases eventually to generate absorbable small peptides and amino acids. Apparently, large protein molecules are not absorbable in intact forms into blood circulation, so production and absorption behavior of digestion peptide metabolites from proteins in blood circulation is a key factor to understand their physiological potentials. Thus, the present study aimed to provide evidence on the appearance of peptide metabolites in bloodstream after protein oral administration and to establish a comprehensive identification method of peptide metabolites by advanced MS approaches.

Firstly, β-conglycinin (100 mg/kg B.W.) was orally administered to Wistar rats (male, 11-week-old) and blood was collected from the tail vein at a time-schedule of 1, 2, 4, and 8 h following administration. MALDI-MS was primarily used for a rough non-target screening of MS signals specific for the blood of β-conglycinin-administered Wistar rats. LC-TOF/MS in combination with peptide synthesis was a good analytical tool to identify peptides, according to marker MALDI-MS signals. Among the 37 signals in β-conglycinin-administered blood by MALDI-MS and LC-TOF/MS analyses, 9 signals were successfully identified as β-conglycinin-derived peptides (i.e., SEL, KGPL, SILGA, DSEL, GDANI, SYFV, CLQSC, GEQPRPF, and LVINEGDA), at plasma concentrations of 0.75–756 pmol/mL. It provided the first evidence to produce oligopeptides in blood circulation from the oral administration of β-conglycinin.

Secondly, Coumarin derivatization method in combination with MALDI-MS was proposed for initial screening of peptide metabolites in rat blood after glycinin administration. Coumarin derivatization can be done at rapid (30 min) and mild (25 °C, pH 8.5) conditions; for synthetic oligopeptides, their MS intensities were enhanced by Cou-derivatization. In addition, MS shifts of

target by Cou moiety (+202.0 *m/z*) allowed an easy detection of peptide metabolites from administered glycinin. In plasma obtained after oral administration of glycinin (100 mg/kg) to SD rats, 15 di- to tetrapeptides were successfully identified as glycinin-derived metabolites. Therefore, the proposed Cou-tagged MALDI-MS and subsequent LC-TOF/MS analysis would be extensively used for overall identification of amine metabolites in the body by their high selective (+ Cou increment) and high sensitive (> 5 pmol/spot) properties. In the perspective, the proposed Cou-tagged MS assays will lead us to elucidate the candidates and mechanism on health-benefits of dietary protein from the aspect of bioactive peptide production behavior in the body system.

In conclusion, the present study has demonstrated that the production of peptide metabolites in rat bloodstream after protein oral administration. Moreover, analytical assay of peptide metabolites in blood by amine derivatization- MALDI-MS technique may be useful for clarifying the systemic absorption and metabolism behavior of bioactive proteins in the body.